PROTEIN METABOLISM AND EXCHANGE AS INFLUENCED BY CONSTRICTION OF THE VENA CAVA*

II. Effects of Parenterally Administered Plasma, Amino Acid Mixture, and Ascitic Fluid, and of Orally Administered Ascitic Fluid in the Experimental Ascitic Dog

By FRANK W. McKEE, M.D., ROBERT E. HYATT, WILLIAM G. WILT, Jr., M.D., GARSON H. TISHKOFF, and GEORGE H. WHIPPLE, M.D.

From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, New York

(Received for publication, July 13, 1949)

Experimental ascites, produced in dogs by placing a constricting aluminum band about the inferior vena cava above the diaphragm, enables the investigator to study many factors related to simple ascites, uncomplicated by abnormalities of the liver, kidneys, or cardiac disease and the related general circulatory difficulties. This simplification presents advantages, but also disadvantages in that no answer is forthcoming to many clinical questions.

Earlier experiments (2, 3) have stressed the importance of protein and sodium chloride in the control of ascitic accumulation in these dogs with supradiaphragmatic vena cava constriction. Consequently we expected to show that high protein levels and stores due to plasma or ascitic fluid by vein would check ascitic production. The exact reverse was noted and the great fluctuations in circulating protein levels associated with rapid formation of ascites are not easy to explain. Sodium chloride without doubt is an important factor, but probably not the only one.

Frequent ascitic fluid removal in these dogs is an "internal plasmapheresis" similar to repeated bleedings and return of washed red cells (simple plasmapheresis). These techniques measure the capacity of the dog to produce proteins (plasma or ascitic) and demonstrate very active formation of these important proteins (60 to 90 gm. per week). Some of these proteins are formed in the liver and this evidence supports other functional tests to indicate a normal liver function.

The rapid fluctuation of the proteins in plasma and ascitic fluid, and the rapid production of ascites in these dogs indicate an easy exchange of proteins between the circulation, tissue spaces, and peritoneal cavity—a dynamic protein equilibrium. It also suggests a circulation of protein-containing fluid between the peritoneal cavity and the general circulation. Ascitic fluid is not a static commodity, but is a protein pool in a constant state of flux—additions due to

* We are indebted to Eli Lilly and Company for aid in conducting this work. We are indebted to Merck and Co., Inc., for valuable amino acids.

some factors, and subtractions due to other factors. Convincing evidence using radio carbon lysine to label plasma proteins will be forthcoming.

Methods

The animals used in these experiments were healthy mongrel dogs maintained on the routine kennel diet of hospital scraps for several months prior to the experiments. After preliminary studies to establish normal plasma protein levels and nitrogen metabolism tendencies in each animal, the constricting band was placed about the vena cava above the diaphragm (2-4).[1]

Ascitic fluid accumulation began rather promptly in all cases on administration of the first course of sodium chloride, a procedure we have accepted as a "standardization" of the efficacy of the operative procedure.

Determinations of plasma protein and ascitic fluid protein were made by the macro-Kjeldahl method. Plasma albumin and ascitic fluid albumin were measured by the methanol precipitation method of Pillemer and Hutchinson (6), followed by a macro-Kjeldahl study of the filtrate. Urinary nitrogen was determined on 48 hour samples by the macro-Kjeldahl method. Fibrinogen was determined by micro-Kjeldahl analysis of the clot following calcium chloride precipitation.

The low protein diet consisted of 100 gm. of a sucrose, lard, bone ash, mixture with added vitamins plus 30 gm. of cooked horsemeat and ferric citrate (600 mg. iron). It had a daily nitrogen content of 1.5 gm.

The high protein diet consisted of 200,250, or 300 gm. of cooked horsemeat (depending on the size and the appetite of the animal fed) and contained 4.46 gm. of nitrogen per 100 gm.

The dog plasma utilized in the experiments was a pooled mixture obtained by daily bleeding of two members of the healthy donor colony. Liquamin Roche (heparin) was used as an anticoagulant (1.2 cc. per 300 cc. whole blood). The blood was collected and centrifuged under sterile conditions, and the plasma was drawn off and administered with sterile equipment. 300 to 400 cc. were given daily and the protein per cent averaged 6.5 gm.

The ascitic fluid was obtained either from dogs used in companion experiments or from the recipient dog itself, thus in some cases autotransfusions were done. This material was likewise collected with sterile technique, but without anticoagulants, and was injected quickly before cooling. 300 to 400 cc. were given daily. The plasma and parenterally administered ascitic fluid were drawn fresh each day. The ascitic fluid used in the oral experiments was obtained each day from a deep-freeze bank, where fluid withdrawn in other experiments was stored in a frozen state until needed. The fluid was thawed and given either by stomach tube in two daily doses of 250 to 350 cc. each, or was mixed with 150 gm. of low protein diet and presented to the animal in a dish. Two animals developed a fondness for this mixture, while a third refused it.

The amino acid mixture was prepared by Merck and Co. and consists of two fractions of a casein hydrolysate fortified with *dl*-tryptophane and *dl*-methionine. It is known as Vuj-N-ix. 20 gm. of this mixture contain the following quantities of these amino acids: Threonine 0.3 gm., valine 1.06 gm., leucine 3.44 gm., isoleucine 1.48 gm., lysine 1.8 gm., tryptophane 0.18 gm., phenylalanine 1.2 gm., methionine 1.7 gm., histidine 0.6 gm., arginine 1.0 gm., glycine 4.92 gm., for a total of 17.68 gm. of amino acids. The remaining 2.32 gm. represent 0.18 gm. of *d*-tryptophane, 0.54 gm. HCl, and 1.6 gm. of unknown constituents which may possibly be non-essential amino acids. Previous experiments in this laboratory (1, 5, 7) have shown this mixture to be effective in plasma protein production. The nitrogen content is 15.0 gm.

[1] We are indebted to Dr. John A. Schilling of the Department of Surgery who performed these operations.

per cent. 20 gm. of this mixture containing 3 gm. of nitrogen were given daily in 150 cc. of either distilled water or normal saline in the two series of experiments (Tables 3 and 4). The mixture was dissolved in distilled water or normal saline, brought to the boiling point, filtered, transferred to a sterile drip bottle, and introduced intravenously with sterile equipment. Mild nausea and some urticarial manifestations were noted usually on administration of the first dose, but no untoward results occurred, and subsequent injections were symptomless.

Nitrogen balance was determined by subtracting nitrogen output (ascitic fluid and urine) from nitrogen intake (diet and/or injected test material).

TABLE 1

Dog Plasma Administered Intravenously Increases Ascitic Fluid

Dog 42-893

Period 7 days	Diet	Weight	Hematocrit	Concentration: Plasma proteins		Concentration: Ascitic fluid proteins		Ascitic fluid removed: Volume	Ascitic fluid removed: Total protein	Fibrinogen	Nitrogen balance: Intake: Plasma and diet	Nitrogen balance: Output: A.F.	Nitrogen balance: Output: Urine	Intake minus output
		kg.	*per cent*	*gm. per cent*	*A/G*	*gm. per cent*	*A/G*	*cc.*	*gm.*	*mg. per cent*	*gm.*	*gm.*	*gm.*	
1	Low protein plus sodium chloride	10.2 10.7	31.5 33.0	4.3 4.1	2.2 1.1	1.9 1.0	— —	2220 1150	42.2 11.5	280 288	—	8.5	10.8	−19.3
2	Whole dog plasma intravenously	10.7 10.1	27.0 28.8	5.1 5.9	0.6 2.3	— 2.7	— 2.1	— 1780	— 48.1	310 295	29.1	7.7	8.0	+13.4
3	Whole dog plasma intravenously	9.0 9.9	27.5 22.1	6.3 7.0	1.3 2.1	4.0 4.7	1.3 2.0	2330 1700	93.2 79.9	— 294	19.7	27.7	6.0	−14.0
4	Low protein	8.8	26.3	6.9	1.0	—	—	—	—	295	7.6	—	3.2	+4.4
	High protein	8.6	26.1	7.5	1.0	—	—	—	—	337	49.1	—	20.3	+28.8
5	High protein	9.3 9.9	27.6 29.0	8.0 7.4	1.0 0.7	— —	— —	— —	— —	352 330	82.1	—	52.1	+30.0
6	High protein	9.7 10.0	29.7 28.8	7.4 7.4	0.9 0.7	4.1 —	1.8 —	870 —	35.7 —	332 —	93.7	5.7	46.6	+41.4

EXPERIMENTAL OBSERVATIONS

Dog plasma (heparinized and given by vein) has a definite but quite unexpected effect. The response is uniform and well shown in Tables 1 and 2. Weight figures have little significance because of ascites fluctuation, formation and withdrawal. Fibrinogen figures are all within normal range. Hematocrits show no significant change but a moderate grade of anemia. Dogs are generally in negative nitrogen balance except during periods of high protein feeding.

This dog had been tested previously (2–4) on various salt-free, salt-containing, high or low protein diets. The low protein diet in Table 1 plus 6 gm. sodium chloride (period 1) produced abundant ascitic fluid with a low protein content and the plasma protein circulating level is 4.3 to 4.1 gm. per cent. Plasma (350 cc. daily) given by vein plus 50 gm. glucose and a vitamin mixture in 300 cc. water by mouth raised the circulating plasma protein levels from 4.1 gm. per cent to 5.9 and 7.0 gm. per cent in successive weeks (periods 2 and 3, Table 1). In the face of these high levels of circulating plasma protein the production of ascitic fluid remained high and the level of contained protein in the ascitic fluid doubled and quadrupled. When the plasma protein by vein was discontinued the production of ascites dropped to very low levels while the levels of the circulating plasma protein increased slightly.

We would emphasize the fact that the A/G ratio of the ascitic fluid ran parallel to that in the circulating plasma (periods 2 and 3, Table 1). There was no great predominance of albumin in the ascitic fluid which must be formed from the plasma or tissue proteins. Evidently globulins cross cell barriers or membranes as easily as albumins under these experimental conditions. A total of 221 gm. protein was removed during periods 2 and 3 (Table 1) while a total of 306 gm. plasma protein was being given by vein. The loss is equal to 72 per cent of the plasma protein given.

Table 2 is very like Table 1 in the response to plasma by vein—about 350 cc. per day. The dog was forming abundant ascitic fluid on the kennel diet. When given plasma by vein and 50 gm. glucose with 10 cc. vitamin mixture in 300 cc. water by mouth, the ascitic fluid increased in volume and its protein content rose. The A/G ratio in circulating plasma and ascitic fluid was very similar (periods 2 and 3, Table 2), and electrophoretic patterns of circulating plasma and ascitic fluid were similar, with lower levels of all proteins being recorded for the ascitic fluid.

When plasma by vein was discontinued the low protein diet was continued for 3 weeks—periods 4, 5, and 6 (Table 2). The production of ascitic fluid decreased sharply. The plasma protein circulating levels fell. Forced fluids (800 cc. water daily by stomach tube) in period 6 produced no significant change. This dog received 321 gm. of plasma protein as plasma and during periods 2 and 3 ascitic fluid proteins 245 gm. were removed—that is 76 per cent as much protein lost in ascitic fluid removal as was given by vein.

A third experiment of similar type was performed. As it was similar to the two experiments tabulated we merely record it as supporting evidence. 301 gm. of plasma protein was given by vein as plasma and 197 gm. of ascitic protein was removed—65 per cent as much as given. In this experiment the A/G ratio of the circulating plasma was 1.0 to 0.7 for the 2 week period while the A/G ratio of the ascitic fluid removed was 1.4 to 0.8.

Amino acids (Tables 3 and 4) present some interesting facts. The growth mixture of amino acids is known to favor the production of blood proteins in dogs with anemia and hypoproteinemia (5). There is a serious weight loss simultaneously which has been discussed elsewhere (5). Table 3 shows two satisfactory experiments in two different dogs. The amino acid mixture is given in solution by vein using distilled water. This procedure checks the formation of ascites

TABLE 2

Dog Plasma Administered Intravenously Increases Ascitic Fluid
Forced Fluid by Mouth Is without Effect

Dog 40-37

Period 7 days	Diet	Weight	Hematocrit	Concentration: Plasma proteins		Concentration: Ascitic fluid proteins		Ascitic fluid removed: Volume	Ascitic fluid removed: Total protein	Fibrinogen	Nitrogen balance: Intake: Plasma and diet	Nitrogen balance: Output: A.F.	Nitrogen balance: Output: Urine	Nitrogen balance: Intake minus output
		kg.	*per cent*	*gm. per cent*	*A/G*	*gm. per cent*	*A/G*	*cc.*	*gm.*	*mg. per cent*	*gm.*	*gm.*	*gm.*	
1	Kennel diet	11.9	43.0	4.6	5.0	—	—	—	—	210	—	—	—	—
		11.9	40.6	4.6	—	3.5	3.6	1950	68.3	193				
2	Whole dog plasma intravenously	12.6	43.1	6.3	2.6	4.8	2.0	1330	63.8	197	26.0	18.1	19.2	−11.3
		12.0	43.4	6.7	1.7	5.3	2.2	930	49.3	210				
3	Whole dog plasma intravenously	12.5	40.8	6.9	1.7	5.2	1.6	1020	53.0	—	25.2	21.1	11.0	−6.9
		12.5	37.6	6.7	1.7	5.1	1.9	1550	79.0	362				
4	Low protein diet	11.8	33.8	5.7	—	—	—	—	—	117	14.3	—	10.8	+3.5
		11.9	37.2	5.5	1.1	—	—	—	—	195				
5	Low protein diet	12.0	39.0	5.6	2.0	3.7	—	930	34.4	225	9.0	5.5	9.2	−5.7
		11.6	36.0	4.9	2.3	—	—	—	—	—				
6	Low protein diet plus forced fluids	11.4	36.4	4.9	1.4	—	—	—	—	282	9.0	3.3	9.6	−3.9
		10.8	38.2	5.2	0.7	3.1	—	675	20.9	270				

and causes a prompt rise in the level of circulating plasma protein (Table 3 periods 3 and 4). In both dogs (Table 3) 20 gm. of the amino acid mixture dissolved in 150 cc. distilled water was given by vein with similar results. Periods 1 and 6 in each dog show an abundant ascitic formation associated with the low protein diet plus 6 gm. sodium chloride daily. Dog 42-893 (Table 3) shows a very low A/G ratio (0.6 to 0.3) during the amino acid periods but the high protein diet corrects this ratio.

Table 4 shows an interesting response to amino acids (20 gm. daily) given

TABLE 3

Amino Acid Mixtures in Distilled Water Administered Intravenously Check Ascites

Dog 40-37

Period 7 days	Diet	Weight	Hematocrit	Concentration: Plasma proteins		Concentration: Ascitic fluid proteins		Ascitic fluid removed: Volume	Ascitic fluid removed: Total protein	Fibrinogen	Nitrogen balance: Intake: Amino acid plus diet	Nitrogen balance: Output: A.F.	Nitrogen balance: Output: Urine	Nitrogen balance: Intake minus output
		kg.	*per cent*	*gm. per cent*	*A/G*	*gm. per cent*	*A/G*	*cc.*	*gm.*	*mg.*	*gm.*	*gm.*	*gm.*	
1	Low protein plus sodium chloride	11.0	44.9	5.7	0.6	2.8	0.9	2000	56.0	364	11.1	16.1	17.6	−22.6
		11.7	45.8	3.6	1.7	1.8	1.7	2500	45.0	283				
2	Low protein plus NaCl	11.7	47.0	3.5	1.1	1.6	1.8	1830	29.3	239	9.4	4.7	20.3	−15.6
										315				
	Low protein	10.3	50.9	4.1	1.0	—	—	—	—					
3	Amino acid mixture intravenously	10.0	42.3	4.8	0.6	—	—	—	—	407	21.0	—	19.2	+1.8
		9.5	39.0	6.0	0.7	—	—	—	—	596				
4	Amino acid mixture intravenously	9.0	38.7	7.1	0.9	—	—	—	—	—	20.1	—	25.0	−4.9
		8.4	39.0	7.5	1.3	—	—	—	—	—				
5	High protein	9.0	36.8	8.1	0.9	—	—	—	—	626	93.7	—	32.9	+60.8
		9.0	38.6	8.6	1.4	—	—	—	—	477				
Dog 42-893														
6	Low protein plus sodium chloride	11.9	33.8	5.2	0.6	2.7	0.9	3130	84.5	268	11.1	17.7	25.6	−32.2
		12.5	38.4	4.9	0.6	1.7	1.3	1500	25.5	—				
7	Amino acid mixture intravenously	10.9	35.3	5.4	0.5	—	—	—	—	298	20.6	—	19.1	1.5
		10.6	32.0	5.9	0.4	—	—	—	—	296				
8	Amino acid mixture intravenously	10.2	32.2	6.4	0.3	—	—	—	—	281	21.0	—	17.6	3.4
		10.0	27.3	6.4	0.4	—	—	—	—	355				
9	High protein	9.5	28.8	6.9	0.5	—	—	—	—	328	71.4	—	32.8	38.6
		10.2	28.1	7.0	0.6	—	—	—	—	—				
10	High protein	10.0	30.9	7.1	1.3	—	—	—	—	326	93.7	—	45.4	48.3

in normal saline by vein. The normal saline (150 cc.) contained 1.35 gm. sodium chloride daily and the response is definite and favors ascitic fluid production, in contrast to the results presented in Table 3. As the experiments in Tables

3 and 4 are otherwise identical it seems very probable that the sodium chloride is the factor which is responsible for the production of ascitic fluid in moderate

TABLE 4

Amino Acid Mixtures in Normal Saline Administered Intravenously Produce Ascites

Dog 42-893

Period 7 days	Diet	Weight	Hematocrit	Concentration: Plasma proteins		Concentration: Ascitic fluid proteins		Ascitic fluid removed: Volume	Ascitic fluid removed: Total protein	Fibrinogen	Nitrogen balance: Intake: Amino acid plus diet	Nitrogen balance: Output: A.F.	Nitrogen balance: Output: Urine	Nitrogen balance: Intake minus output
		kg.	*per cent*	*gm. per cent*	*A/G*	*gm. per cent*	*A/G*	*cc.*	*gm.*	*mg. per cent*	*gm.*	*gm.*	*gm.*	
1	High protein	10.3	36.1	5.8	—	3.7	1.4	550	20.4	322	65.2	3.2	42.7	+19.3
		10.3	40.2	7.1	0.7	—	—	—	—	—				
2	Amino acid in saline intravenously	11.6	38.5	6.0	0.7	3.6	0.7	390	14.0	412	21.0	4.1	21.9	−5.0
		11.4	32.5	5.9	0.9	2.4	1.1	490	11.8	374				
3	Amino acid in saline intravenously	10.4	33.8	6.1	0.7	2.6	2.8	110	2.9	460	9.0	0.4	11.6	−3.0
	High protein	10.2	29.0	6.7	0.6	—	—	—	—	475	17.9	—	11.8	+6.1
4	High protein	10.4	32.6	6.8	0.9	—	—	—	—	450	85.9	—	56.7	+29.2
		10.4	34.1	7.6	1.0	—	—	—	—	520				
Dog 47-194														
5	High protein	9.3	55.8	5.4	—	—	—	—	—	145	78.1	—	48.6	29.5
		9.0	53.5	6.0	—	—	—	—	—	148				
6	Amino acid in saline intravenously	10.0	50.7	4.1	3.6	3.8	0.8	800	30.4	228	19.6	8.2	28.3	−16.9
		10.0	44.7	4.5	1.7	2.8	1.0	750	21.0	137				
7	Amino acid in saline intravenously	9.3	43.8	4.2	2.4	2.6	1.2	450	11.7	205	21.0	5.7	19.4	−4.1
		9.7	47.1	4.1	1.7	2.6	2.2	920	23.9	178				
8	High protein	9.0	46.7	5.1	1.3	—	—	—	—	254	71.5	—	35.0	36.5
		9.0	55.2	6.0	3.5	—	—	—	—	184				

abundance (Table 4). In period 1 (Table 4) dog 42-893, we note removal of 550 cc. of ascitic fluid—a carry-over from the preceding 4 week high protein period.

As the ascitic fluid accumulates the level of circulating plasma protein falls —this fall being greater in the second experiment where more ascitic fluid was produced. Of particular interest are the A/G ratios during the amino acid injection periods (2 and 3, Table 4). Some of these periods show lower A/G ratios in the ascitic fluid than in the circulating plasma, again an indication that

TABLE 5

Ascitic Fluid Administered Intravenously Produces Ascites

Dog 42-893

Period 7 days	Diet	Weight	Hematocrit	Concentration: Plasma proteins		Concentration: Ascitic fluid proteins		Ascitic fluid removed: Volume	Ascitic fluid removed: Total protein	Fibrinogen	Nitrogen balance: Intake (Ascitic fluid and diet)	Nitrogen balance: Output A.F.	Nitrogen balance: Output Urine	Intake minus output
		kg.	*per cent*	*gm. per cent*	*A/G*	*gm. per cent*	*A/G*	*cc.*	*gm.*	*mg. per cent*	*gm.*	*gm.*	*gm.*	
1	High protein	10.7	35.5	6.3	0.8	—	—	—	—	302	73.6	—	71.4	2.2
		10.9	32.1	6.0	0.6	—	—	—	—	237				
2	Ascitic fluid intravenously	10.9	33.3	6.9	0.9	—	—	—	—	266	11.9	6.8	16.5	−11.4
		10.9	35.3	5.9	0.9	3.4	—	1250	42.5	184				
3	Ascitic fluid intravenously	10.9	36.4	5.7	0.6	3.4	—	890	30.2	104	13.8	22.6	17.3	−26.1
		10.7	—	5.6	0.5	3.6	—	3090	111.2	63				
4	High protein	8.6	38.6	7.4	0.9	—	—	—	—	302	93.7	—	50.7	43.0
		8.6	39.8	6.4	0.7	—	—	—	—	—				
5	High protein	8.9	41.6	7.1	0.6	—	—	—	—	—	88.3	—	79.0	9.3
		9.3	41.3	6.9	1.0	—	—	—	—	312				
Dog 48-33														
6	High protein	7.3	41.8	6.5	2.4	—	—	—	—	93	61.4	—	53.2	8.2
		7.7	37.6	6.6	1.5	—	—	—	—	—				
7	Ascitic fluid intravenously	7.7	36.4	6.1	0.8	—	—	—	—	98	12.7	15.2	18.9	−21.4
		8.5	33.8	5.4	0.5	3.9	0.9	2430	94.8	—				
8	Ascitic fluid intravenously	7.7	37.0	5.5	1.7	—	—	—	—	90	13.7	10.6	13.0	−9.9
		8.0	34.0	5.7	0.7	3.6	2.2	1840	66.2	143				
9	High protein	6.3	44.1	7.2	1.0	—	—	—	—	117	78.1	—	46.3	31.8
		6.3	43.1	7.0	1.3	—	—	—	—	—				

the globulins appear in abundance in the peritoneal cavity. Evidently these globulins of considerable molecular size cross cell or membrane borders with ease, whatever the mechanism of transfer may be.

Ascitic fluid has been given intravenously at times to supply protein to patients and under certain conditions the material can in all probability be utilized. In these dog experiments (Table 5) the response was unfavorable, probably due to the sodium chloride contained in the ascitic fluid. These specimens contained only ½ to ¼ as much proteins as the normal plasma but presumably the same amount of sodium chloride—in other words more sodium per gram of protein.

TABLE 6

Ascitic Fluid Given Orally Produces Ascites and Hypoproteinemia

Dog 47-194

Period 7 days	Diet	Weight	Hematocrit	Concentration: Plasma proteins		Concentration: Ascitic fluid proteins		Ascitic fluid removed: Volume	Ascitic fluid removed: Total protein	Fibrinogen	Nitrogen balance: Intake: Ascitic fluid and diet	Nitrogen balance: Output: A.F.	Nitrogen balance: Output: Urine	Nitrogen balance: Intake minus output
		kg.	*per cent*	*gm. per cent*	*A/G*	*gm. per cent*	*A/G*	*cc.*	*gm.*	*mg. per cent*	*gm.*	*gm.*	*gm.*	
1	High protein	9.5	51.4	6.4	1.7	—	—	—	—	216	78.0	—	32.7	45.3
		9.8	51.8	6.2	1.4	—	—	—	—	220				
2	Ascitic fluid orally	9.5	51.9	3.9	3.5	3.5	—	1850	64.8	194	24.6	18.0	32.7	−26.1
		9.5	56.5	2.9	2.0	2.1	5.0	2270	47.7	—				
3	Ascitic fluid orally	10.4	43.7	3.5	1.8	1.8	—	1785	32.2	250	10.5	5.2	6.2	−0.9
	High protein	9.5	45.4	4.6	0.9	—	—	—	—	214	41.5	—	17.1	24.4
4	High protein	9.3,	53.4	4.9	1.5	—	—	—	—	208	78.0	—	43.8	34.2
		9.3	53.0	5.2	1.5	—	—	—	—	130				
5	High protein	9.4	54.0	5.5	2.5	—	—	—	—	124	33.5	—	18.0	15.5
6	Ascitic fluid orally	8.9	59.0	3.4	2.0	3.0	1.0	2440	73.2	210	21.6	15.7	21.8	−15.9
		9.3	57.0	3.3	1.1	1.6	3.4	1550	24.8	202				
7	Ascitic fluid orally	10.7	47.7	3.6	4.4	1.5	—	1610	24.2	177	13.1	7.0	9.2	−3.1
		9.7	47.5	3.6	1.0	0.9	—	2150	19.4	216				
	High protein	8.9	47.0	4.6	0.6	1.4	2.8	930	13.0	—	51.2	2.1	22.6	26.5

Table 5 shows two satisfactory experiments. Ascites appeared in abundance when ascitic fluid was given intravenously. In dog 42-893 (Table 5) the donor ascitic fluid 300 to 350 cc. given daily caused a large outpouring of ascitic fluid and a drop in circulating plasma protein levels. This dog received 159 gm. of protein in ascitic fluid by vein and lost 184 gm. in the removed ascitic fluid. A high protein diet returned the dog to balance and terminated production of ascitic fluid. Dog 48-33 in Table 5 shows a similar response. The dog received 164 gm. of protein in ascitic fluid by vein and lost 161 gm. in the removed ascitic fluid.

Tables 6 and 7 show the response to feeding of ascitic fluid to these dogs. The response is remarkable and deserves much more study. Table 6 shows a sharp drop in circulating plasma protein levels from 6.4 to 6.2 to 2.9 to 3.5 gm. per cent in periods 1, 2, and 3 when the high protein diet is replaced by a low protein diet plus 600 cc. ascitic fluid containing 20 to 25 gm. protein daily. The total intake of ascitic protein by mouth was 220 gm. and the total removed as ascitic fluid was 145 gm. Other experiments with large protein loss through ascites (low protein diet plus salt) did not show as low levels of circulating plasma protein. The hematocrits gave no evidence of plasma dilution—if anything slight concentration. The dog was lively and normal and there was no evidence of tissue edema. It would appear that the ascitic fluid fed contained something beside sodium chloride which favors a rapid escape of plasma proteins—this observation therefore touches some of the fundamental mechanisms of the dynamic equilibrium of body proteins.

TABLE 7

Ascitic Fluid Given Orally Produces Ascites and Hypoproteinemia

Dog 48-33

Period 7 days	Diet	Weight	Hematocrit	Concentration: Plasma proteins		Concentration: Ascitic fluid proteins		Ascitic fluid removed: Volume	Ascitic fluid removed: Total protein	Fibrinogen	Nitrogen balance: Intake (Ascitic fluid and diet)	Nitrogen balance: Output A.F.	Nitrogen balance: Output Urine	Nitrogen balance: Intake minus output
		kg.	per cent	gm. per cent	A/G	gm. per cent	A/G	cc.	gm.	mg. per cent	gm.	gm.	gm.	
1	High protein	6.3	44.1	7.2	1.0	—	—	—	—	117	78.1	—	46.3	31.8
		6.3	43.1	7.0	1.3	—	—	—	—	—				
2	High protein	6.4	48.1	7.0	1.4	—	—	—	—	—	79.1	—	49.0	30.1
		6.9	45.4	7.1	0.9	—	—	—	—	—				
3	Ascitic fluid orally	9.1	47.5	4.9	1.6	2.4	1.7	2430	58.3	—	23.5	15.0	18.8	−10.3
		9.5	48.5	4.3	1.3	1.2	—	2970	35.6	250				
4	Ascitic fluid orally	8.4	48.1	4.4	1.0	1.2	1.9	2010	24.1	274	21.3	8.9	17.8	−5.4
		9.7	45.2	4.3	0.8	1.0	1.4	3040	30.4	190				
5	High protein	7.0	43.9	5.7	0.6	—	—	—	—	354	63.0	—	23.5	39.5
		7.3	46.0	6.5	1.1	—	—	—	—	356				

When a high protein diet replaced the ascitic fluid diet the plasma protein levels rise—nitrogen balance again became positive, weight balance returned to normal. A similar response appears in periods 5, 6, and 7 when ascitic fluid and low protein diet were again given. We note the same general reaction as above (periods 2 and 3).

The A/G ratios in the removed ascitic fluid show wide fluctuations. This is in harmony with rapid accumulation and wide variations in the total protein of the ascitic fluid. If the ascitic fluid is being constantly absorbed and constantly produced, these changes are not surprising. Evidence is at hand in experiments in this laboratory that there is in truth a rather rapid circulation of the ascitic fluid—perhaps a total turnover of the ascitic pool within 5 to 7 days in certain experiments using radio carbon lysine to label the plasma proteins or the ascitic proteins.

Table 7 shows an experiment similar to that in Table 6. Ascitic fluid was given orally (600 to 700 cc. daily containing 20 to 25 gm. of protein) mixed with 150 gm. of the low protein diet, and was eaten readily. Note the sharp fall in circulating levels of plasma protein from 7.0 to 4.3 gm. per cent. Abundant ascitic fluid appeared and was removed from the peritoneal cavity. During the 2 week period the dog received 276 gm. ascitic fluid protein by mouth and produced 148 gm. ascitic fluid protein. High protein diet before and after periods 3 and 4 corrects the conditions noted above and controls the ascites.

DISCUSSION

As sometimes happens, the reactions following these experiments (plasma injections) were quite contrary to our expectations. We had the conviction that a high level of circulating plasma protein would check ascites just as a high protein diet will do in these dogs.

The reaction to ascitic fluid given by vein is an abundant production of ascitic fluid low in protein. The response gives a larger volume of ascitic fluid than was observed following plasma by vein.

Ascitic fluid fed to these dogs in considerable amounts (500 to 600 cc. daily containing 20 to 25 gm. of protein) gives an even more striking response, large production of new ascitic fluid and a great drop in circulating plasma protein levels (from a control of 7.0 to 6.0 gm. per cent to 4.0 to 3.0 gm. per cent) within a few days. Evidently ascitic fluid contains something besides sodium chloride which favors the production of ascitic fluid in these dogs.

Amino acids in water cause no ascites but when given in normal saline (0.9 per cent) by vein they effect the production of a moderate grade of ascites. This points up to the importance of sodium chloride which will cause ascites in the face of rising plasma protein levels. Evidently sodium chloride is a factor which dominates the situation, in these experiments being more important than the protein levels. It would appear probable to us that ascitic fluid does not contain enough sodium chloride to explain the profound response noted above (Tables 5 to 7). This point deserves further study.

The A/G ratios are of considerable interest because in the tables above the ratio is frequently lower in the ascitic fluid than in circulating plasma. This means abundant globulins in the ascitic fluid, and indicates ready transfer of the larger globulin molecules across cell barriers or membranes. Electrophoretic patterns of the ascitic fluid and the blood plasma are quite similar (2) except for the fibrinogen peak which is lower in the ascitic fluid, probably due to coagulation of ascitic fluid and autolysis of the soft clots. Obviously a salt-free albumin or globulin would be useful for subsequent tests in these ascitic dogs. Foreign proteins cannot be used to test any normal reactions, but eventually we hope to use salt-free dog plasma in these experiments.

It is to be emphasized that these dogs are suffering from stasis within the portal area, but the response is not complicated by significant abnormality of liver structure or function. There is no complicating nephritis, heart disease, arteriosclerosis, or gastrointestinal abnormality (except stasis) to confuse the response. Therefore these observations cannot be compared directly with those reported in many of the complicated human cases with multiple abnormalities.

SUMMARY

Further studies of ascitic fluid production and related factors in dogs with constriction of the vena cava above the diaphragm are reported.

Whole dog plasma given intravenously to such animals produces a rise in circulating plasma protein to normal levels, but increases the output of ascitic fluid with a loss of protein *via* the ascites equivalent to 72, 76, and 65 per cent respectively, of the injected protein.

Forced ingestion of water in excess of the test animal's normal needs and desires produces no significant changes in the circulating plasma protein level or in ascitic fluid production.

Amino acid growth mixtures given intravenously in distilled water cause weight loss, elevation of circulating plasma proteins, a slightly negative nitrogen balance, but no ascitic fluid production.

Amino acid growth mixtures given intravenously in normal saline cause depression of the circulating plasma proteins, negative nitrogen balance, and significant ascitic fluid production.

Ascitic fluid given intravenously to the test animals causes a marked depression of circulating plasma proteins, a marked increase in ascitic fluid production containing the equivalent of 116 and 98 per cent of the injected protein, and a negative nitrogen balance.

Ascitic fluid given orally produces a marked depression of circulating plasma proteins, and a marked increase in ascitic fluid secretion, containing the equivalent of 66, 66, and 54 per cent respectively, of the ingested protein.

Sodium chloride is a dominant factor in some of these experiments where abundant ascites production is recorded. Protein levels and intake are important, but take second place to sodium.

Ascitic fluids show electrophoretic patterns which are almost identical to the plasma patterns. The A/G ratios are often equal in ascitic fluid and plasma, sometimes even lower in the ascitic fluid. This emphasizes the ease with which globulins pass cell or other membrane barriers in these experiments.

BIBLIOGRAPHY

1. Madden, S. C., and Clay, W. A., *J. Exp. Med.*, 1945, **82,** 65.
2. McKee, F. W., Schilling, J. A., Tishkoff, G. H., and Hyatt, R. E., *Surg., Gynec. and Obst.*, 1949, in press.
3. McKee, F. W., Schloerb, P. R., Schilling, J. A., Tishkoff, G. H., and Whipple, G. H., *J. Exp. Med.*, 1948, **87,** 457.
4. McKee, F. W., Schloerb, P. R., and Tishkoff, G. H., *Fed. Proc.*, 1947, **6,** 396.
5. Miller, L. L., Robscheit-Robbins, F. S., and Whipple, G. H., *J. Exp. Med.*, 1947, **85,** 267.
6. Pillemer, L., and Hutchinson, M. C., *J. Biol. Chem.*, 1945, **158,** 299.
7. Robscheit-Robbins, F. S., Miller, L. L., and Whipple, G. H., *J. Exp. Med.*, 1947, **85,** 243.